

**RADIOACTIVE MAGNETIC FLUIDS FOR TREATMENT OR
DIAGNOSIS OF CANCER, PROCESS FOR PREPARING THEM AND USE
THEREOF**

5 **Technical Field**

 The present invention relates to radioactive magnetic fluids, process for preparing them and use thereof. Particularly, the present invention relates to the radioactive magnetic fluids for treatment or diagnosis of
10 cancer, process for preparing them and use thereof.

Background of the Invention

 Cancer is a significant problem in human health all over the world. At present, there are a number of methods
15 and techniques for the treatment of cancer. A safe and effective cancer treatment has been the goal of investigators for a substantial period time. A technique for the successful cancer treatment must ultimately differentiate cancer cells from normal cells and must
20 selectively weaken or kill only the cancer cells without affecting the normal cells. Some cancers such as eye cancer and liver cancer cannot be completely treated by a surgical operation.

25 Chemical therapy, radiate therapy and surgical

operation are developed for the treatment of cancer.

In chemical therapy, a chemical material is administered to a body, which kills the cancer cell.

In surgical operation, the affected part of cancer is removed or dressed.

In radiate therapy, the radioactive element which radiates a β (beta)-ray is used for treatment of cancer. The radiate therapy is divided into outer and inner radiate therapies. Outer radiate therapy is to kill the cancer cells by the β -ray radiated from outside. However, the disadvantage of outer radiate therapy is that the treatment efficiency is low and the side effect is too large because the radiation kills the cancer cell during treatment and at the same time destroys a lot of normal cells as well. Inner radiate therapy is to inject radioactive magnetic fluids into the body, and to kill the cancer cell by the β -ray radiated from the radioactive magnetic fluids under external magnetic field.

Particularly, in the inner radiate therapy, magnetic nanoparticles prepared with elements emitting the β -ray are coated with the surfactants to form the magnetic fluids which are injected into the body. Under external magnetic field, the β -ray radiated from the magnetic fluids is positioned around the affected part to kill the tumor cells.

The magnetic fluids used in inner radiate therapy is magnetic colloidal liquid prepared by the steps: 1) coating the magnetic powder with various surfactants, in which the magnetic powder is magnetic oxides such as $MO \cdot Fe_2O_3$ (M :
5 metal cation of 2+) with a mean size of 100\AA ; and 2) dispersing homogeneously and stably the said coated particles into a liquid.

The magnetic fluids as colloidal particles in a suspension are not agglomerated or precipitated by gravity
10 or magnetic force.

U.S. Pat. No. 5921244 suggests the process of magnetic fluids containing the particles coated with photoreactive agent. However, it is restricted within the purpose of drug therapy by a light source using a magnet inserted into the
15 body. Also, U.S. Pat. No. 4957481 deals with the effective amount and direct injection of a photosensitive compound into tumor, and the application and activation of light source.

20 **Disclosure of the Invention**

It is an object of the present invention to provide the radioactive magnetic fluids for treatment or diagnosis of cancer.

It is another object of the present invention to
25 provide the process for preparing the radioactive magnetic

fluids.

It is another object of the present invention to provide the use of the radioactive magnetic fluids.

5 Brief Description of the Drawings

The above and other objects, features and advantages of the present invention will be more clearly understood from the following detailed description taken in conjunction with the accompanying drawings, in which:

10 Fig. 1 is a flow chart diagram of the steps for preparation of the radioactive magnetic fluids of the present invention;

Fig. 2 shows a principle of treatment on a human body by radiate therapy using the radioactive magnetic fluids of
15 the present invention;

Fig. 3 shows a typical X-ray diffraction pattern for the nanoparticles of magnetite(Fe_3O_4) with a copper content of 10%;

Fig. 4 shows magnetization curves for magnetic
20 nanoparticles of $\text{Cu}_x\text{Fe}_{1-x}\text{O} \cdot \text{Fe}_2\text{O}_3$ with different Cu^{2+} contents;

Fig. 5 shows concentration of magnetic fluids, magnetization of nanoparticles and atomic fraction of copper about the magnetic nanoparticles obtained with different precipitators; and

25 Fig. 6 shows magnetization values of magnetic

nanoparticles prepared at various mole ratios of ($\text{Cu}^{2+} + \text{Fe}^{2+}$) to Fe^{3+} .

Best Mode for Carrying Out the Invention

5 The present invention provides radioactive magnetic fluids for treatment or diagnosis of cancer. Particularly, the present invention provides radioactive magnetic fluids comprising: magnetic nanoparticles; and surfactants coated onto the magnetic nanoparticles, wherein the radioactive
10 copper is a component part of the magnetic nanoparticles.

 Preferably, the present invention provides the radioactive magnetic fluids, wherein the magnetic nanoparticles are obtained with synthesis of $\text{Cu}_x\text{Fe}_{1-x}\text{O} \cdot \text{Fe}_2\text{O}_3$
15 by a chemical reaction of the Cu^{2+} component with the components of Fe^{2+} and Fe^{3+} .

 More preferably, the present invention provides the radioactive magnetic fluids, wherein the surfactants
20 comprise the first surfactant coated onto the surface of the magnetic nanoparticles, the first surfactant being decanoic acid, and the second surfactant coated onto the first-coated magnetic nanoparticles, the second surfactant being nonanoic acid.

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Wherein, x value of $\text{Cu}_x\text{Fe}_{1-x}\text{O}\cdot\text{Fe}_2\text{O}_3$ in a chemical composition of the magnetic nanoparticles is between 0.1 and 0.4.

5 The magnetic nanoparticles of $\text{Cu}_x\text{Fe}_{1-x}\text{O}\cdot\text{Fe}_2\text{O}_3$ are prepared by a one-step method, wherein Cu^{2+} is precipitated simultaneously with Fe^{2+} and Fe^{3+} . Particularly, the colloidal particles of Fe_3O_4 are prepared by coprecipitating the solutions of Fe^{2+} and Fe^{3+} . Thereafter,
10 Cu^{2+} is incorporated into the magnetite crystal by substituting Fe^{2+} from the Fe_3O_4 .

The copper element is a radionuclide emitting the β - ray with a energy of 0.577MeV and a half time of 61.83h.
15 The said β -ray penetrates through several millimeters of the body tissue with a short effective range. Therefore, the radioactive magnetic fluids of the present invention can be used for treatment of cancer with minimal damage to normal tissue.

20

Also, the copper element radiates γ (gamma)-ray of 150 keV close to the energy of $^{99\text{m}}\text{Tc}$ which is often used in tumor diagnosis[Urs Hafeli, Gayle Pauer, Sarah Failing, Gilles Tapolsky, "Radiolabeling of magnetic particles with
25 rhenium-188 for cancer therapy", *Journal of Magnetism and*

Magnetic materials, 255, 2001, 73-78]. Since the said γ radiation is easily imaged with γ -camera, the magnetic fluid can be gathered to the treatment site with the radiograph under external magnetic field[Ch. Alexiou, A. Schmidt, R. Klein, P. Hulin, Ch. Bergemann, W. Arnold, "Magnetic drug targeting: biodistribution and dependency on magnetic field strength", *Journal of Magnetism and Magnetic Materials*, 252, 2002, 363-366].

Therefore, the radioactive magnetic fluids of the present invention which are injected into the body through artery can be immobilized at the affected part by imaging with γ -camera under external magnetic field. The magnetic field system gathers the magnetic fluids to create an optimum radiation intensity developed on the desired part of the organism, keeping the total injected dose low. The radioactive magnetic fluids of the present invention kill the tumor cells with little damage to the normal tissue following by minimizing side effects. The radioactive magnetic fluids can be also positioned in the fine organism such as liver and internal organs, to treat the affected part.

Atomic radius, electronegativity and valence of the Cu element are quite similar to those of the Fe element. Since Cu^{2+} can substitute Fe^{2+} from the Fe_3O_4 according to

Hume-Rothery rule, the Cu^{2+} is substitutionally bonded in the oxide crystal. Therefore, the radioactive magnetic fluids can be stably delivered to the affected part, to become both carrier and drug to be bifunctional agent.

5

Generally, in radiate therapy, the dosage of radioactivity for the treatment of cancer keeps 40-100MBq (Bq means one atom disintegration per second). It indicates that the mass of radioactive Cu^{2+} disintegrated per second is $4.89\sim 12.22 \times 10^{-11}$. Although only a few Fe^{2+} from the said Fe_3O_4 are substituted by Cu^{2+} , the prepared magnetic fluids can have both desirable radioactivity and sufficient magnetization.

15 The decanoic acid or nonanoic acid acts as surfactants. Particularly, a functional group of the acid is adsorbed onto the surface of the magnetic nanoparticles prepared by chemical coprecipitation, to form a monomolecular layer around the said particles. The tightly
20 bonded decanoic acid or nonanoic acid layer does not only increase particle-particle repulsion but also takes hydrophobicity or hydrophilicity, to disperse homogeneously and stably the magnetic nanoparticles in the liquid medium. Also, carboxylic acid of the surfactant is exposed to the
25 outside, to prevent the magnetic nanoparticles from being

oxidized by air.

Since the decanoic acid or nonanoic acid is biodegradable fatty acid, the biodegradable magnetic particles coated with the fatty acid can eventually be incorporated into the subject's hemoglobin. Thus, the magnetic particles of the present invention are well-suited for in vivo use.

Also, the present invention provides the process for preparing the radioactive magnetic fluids. Particularly, the process of the present invention comprises:

1)preparing the magnetic nanoparticles of $\text{Cu}_x\text{Fe}_{1-x}\text{O}\cdot\text{Fe}_2\text{O}_3$ by coprecipitating components of Fe^{2+} and Fe^{3+} with a component of Cu^{2+} under the presence of precipitator(step 1);

2)first coating the magnetic nanoparticles with decanoic acid(step 2); and

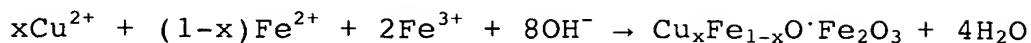
3)second coating the first-coated magnetic nanoparticles with nonanoic acid(step 3).

(step 1)

As shown in the scheme 1, the magnetic nanoparticles of $\text{Cu}_x\text{Fe}_{1-x}\text{O}\cdot\text{Fe}_2\text{O}_3$ is prepared by coprecipitating the components of Fe^{2+} and Fe^{3+} together with the component of

Cu²⁺ under the presence of precipitator.

<scheme 1>



5

The coprecipitation is the process that the mixed solution of Fe²⁺, Fe³⁺ and Cu²⁺ is heated and stirred at the temperature of 80°C. In the said process, Fe₃O₄ is synthesized from the solutions of Fe²⁺ and Fe³⁺, and Cu²⁺ is incorporated into the crystal of magnetite by substituting Fe²⁺ from the said Fe₃O₄.

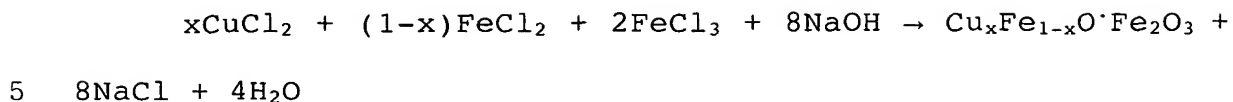
Also, the precipitator is preferable sodium hydroxide. In the case of ammonia water, the excess reacts with Cu(OH)₂ to produce the chelate compound of [Cu(NH₃)₄]²⁺. Therefore, the excess ammonia must be removed in the washing process. The mixture is vigorously agitated in order to restrict the growth of particles.

Also, the mole ratio of (Cu²⁺+Fe²⁺) to Fe³⁺ is within range of (1.1~1.4):2.

The starting materials for Fe²⁺ and Fe³⁺ are preferable iron(II) chloride tetrahydrate(FeCl₂·4H₂O) and iron(III) chloride hexahydrate(FeCl₃·6H₂O), respectively. And the starting material for Cu²⁺ is preferable copper(II) chloride dehydrate(CuCl₂·2H₂O). In case of the above, the

reaction of the step 1 is shown in scheme 2.

<scheme 2>



(wherein, x value of $\text{Cu}_x\text{Fe}_{1-x}\text{O}\cdot\text{Fe}_2\text{O}_3$ in a chemical composition of magnetic nanoparticles is between 0.1 and 0.4.)

10 The magnetic nanoparticles are prepared with the mean size of about 10 nanometers by the said chemical coprecipitation.

(step 2)

15 The magnetic nanoparticles prepared in the above step 1 are first coated with decanoic acid.

Particularly, decanoic acid is added to the said magnetic nanoparticles prepared in the above step 1, thereafter the resulting mixture is stirred for 30min and
20 heated to 80°C. Thus, decanoic acid is chemisorbed on the surface of the magnetic nanoparticles.

(step 3)

The first-coated magnetic nanoparticles prepared in
25 the above step 2 are secondly coated with nonanoic acid.

Particularly, nonanoic acid is added to the said first-coated magnetic nanoparticles prepared in the above step 2, thereafter the resulting mixture is stirred for 30min and heated to 60°C. Thus, water-based magnetic fluids are prepared by immobilizing nonanoic acid.

Fig. 1 is a flow chart diagram of the process for preparing the radioactive magnetic fluids according to the present invention. As shown in the Fig. 1, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (0.148g) and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (1.548g) and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (4.7g) are dissolved in water. The mixed solution is stirred and heated to 80°C. The nano-sized particles are prepared with the addition of NaOH to the solution. Decanoic acid is used as the first surfactant, which is dissolved in solvent. The resulting liquid is added to the said nano-sized particles. The mixture is stirred and heated, for the said nano-sized particles to be first coated with decanoic acid, thereafter the mixture is cooled to the room temperature, for the coated decanoic acid not to break away from the magnetic nanoparticles. The excess decanoic acid is removed by washing with acetone. Subsequently, the remaining acetone is removed by washing with water. Nonanoic acid as the second surfactant is used with a small amount of NaOH. The resulting liquid is added to the said first-coated particles. The mixture is stirred and heated for the first-

coated particles to be secondly coated with nonanoic acid.

Fig. 2 shows a principle of treatment on a human body by radiate therapy using the radioactive magnetic fluid of this invention.

The radioactive magnetic fluids are injected into the body through artery. As shown in Fig. 2, the radioactive magnetic fluids are immobilized at the affected part by an external magnetic field, radiating β -ray and γ -ray for half time. The radiated β -ray can treat the cancer by killing the tumor cell. Since the radiated γ -ray is easily imaged with γ -camera, the magnetic fluids can be gathered to the treatment site with the radiograph under external magnetic field.

Therefore, the radioactive magnetic fluids of the present invention can be used for treatment or diagnosis of cancer as therapeutic drug or diagnostic reagent for cancer.

Also, the radioactive magnetic fluids according to the present invention can be applied in biomedical application such as drug delivery and cell separation. In case of drug delivery, for example, drug can be adsorbed onto the surface of the radioactive magnetic fluids. Thereafter, the said radioactive magnetic fluids can be delivered to the affected part using an external magnetic

field system.

The present invention will be explained in more detail with reference to the following examples in conjunction with the accompanying drawings. However, the following examples are provided only to illustrate the present invention, and the present invention is not limited to them.

<EXAMPLE 1>

FeCl₂·4H₂O 1.548g, FeCl₃·6H₂O 4.7g and CuCl₂·2H₂O 0.148g were dissolved in 80ml of distilled water. The mixed solution was poured into a flask with three ports, was heated to 80°C and was stirred at 300 rpm. About 2g of magnetic nanoparticles was prepared by adding aqueous sodium hydroxide 10ml(sodium hydroxide 6g contained)to the mixed solution and also stirring.

2.4g of decanoic acid was dissolved in acetone 60ml, to prepare the decanoic acid solution. The said decanoic acid solution was added to the said magnetic nanoparticles in six stages. The resulting mixture was stirred and heated, for the said magnetic nanoparticles to be first coated with decanoic acid, was cooled to the room temperature, and was washed with acetone and water.

Thereafter, 3ml of nonanoic acid and 2ml of NH₄OH were added to the first-coated magnetic nanoparticles. The

resulting mixture was stirred for 30min and heated to 60°C, and was cooled to the room temperature, to prepare the radioactive water-based magnetic fluids.

5 <EXPERIMENTAL EXAMPLE 1>

Magnetization of the radioactive magnetic fluids

Whether Cu^{2+} substituted Fe^{2+} of Fe_3O_4 was observed by X-ray diffraction of the magnetic nanoparticles prepared in the example 1.

10 The result is shown in Fig. 3.

In Fig. 3, the vertical bold lines are the standard diffraction pattern for magnetite(Fe_3O_4).

As shown in Fig. 3, intensity and position of the standard diffraction peaks were considerably changed by the
15 incorporation of Cu^{2+} into the magnetite. The result revealed that a ferromagnetic Fe^{2+} of the magnetite was in part substituted by a diamagnetic Cu^{2+} . Particularly, ionic radius of Cu^{2+} (0.069nm) is smaller than that of Fe^{2+} (0.076nm). Therefore, positions of the diffraction
20 peaks for the radioactive magnetic nanoparticles are shifted to the right due to the increase of diffraction angles from Bragg's law, compared with the standard diffraction pattern of magnetite.

25 <EXPERIMENTAL EXAMPLE 2>

Magnetization of the radioactive magnetic nanoparticles with different contents of Cu^{2+}

Magnetization of the radioactive magnetic nanoparticles was measured with different contents of Cu^{2+} by the following experiment.

The contents of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ are represented in table 1. Particularly, with the constant quantity of 4.700g for $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.148g, 0.296g, 0.445g and 0.593g of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ were added to 1.548g, 1.376g, 1.204g and 1.032g of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, respectively. According to the process of example 1, the magnetic nanoparticles of $\text{Cu}_x\text{Fe}_{1-x}\text{O} \cdot \text{Fe}_2\text{O}_3$ were prepared with the above mixed compounds.

Using the magnetic nanoparticles prepared as samples, the value of x for $\text{Cu}_x\text{Fe}_{1-x}\text{O} \cdot \text{Fe}_2\text{O}_3$ was measured by atomic absorption spectroscopy(AA). The results are also listed in table 1.

Table 1

Content	$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$	$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	x value of $\text{Cu}_x\text{Fe}_{1-x}\text{O} \cdot \text{Fe}_2\text{O}_3$
Sample 1	0.148	1.548	4.7	0.1
Sample 2	0.296	1.376	4.7	0.2
Sample 3	0.445	1.204	4.7	0.3
Sample 4	0.593	1.032	4.7	0.4

As shown in table 1, the x value of $\text{Cu}_x\text{Fe}_{1-x}\text{O} \cdot \text{Fe}_2\text{O}_3$ for the magnetic nanoparticles became in turn 0.1, 0.2, 0.3 and 0.4 with increasing copper contents in the samples. Also,

the magnetization of magnetic nanoparticles prepared with the above samples was measured using vibrating sample magnetometer(VSM).

The results are shown in Fig. 4, wherein $\text{Cu}_{0.1}$, $\text{Cu}_{0.2}$,
5 $\text{Cu}_{0.3}$ and $\text{Cu}_{0.4}$ indicate the Cu fractions for $\text{Cu}_x\text{Fe}_{1-x}\text{O}\cdot\text{Fe}_2\text{O}_3$ of magnetic nanoparticles.

As shown in Fig. 4, the magnetization of the magnetic nanoparticles decreased with increasing Cu^{2+} contents. The reason is that the amount of the ferromagnetic phase
10 decreases in the magnetic nanoparticles while the diamagnetic Cu^{2+} substitutionally occupies the site of Fe^{2+} in the crystal structure of Fe_3O_4 .

<EXPERIMENTAL EXAMPLE 3>

15 **Effect of precipitator on characteristic of magnetic fluids**

Concentration of magnetic fluid, magnetization of magnetic nanoparticle and atomic fraction of copper about the magnetic nanoparticles prepared with different
20 precipitators were observed by the following experiment.

Ammonia water or sodium hydroxide was used as precipitator, in the amount of 10ml and 15ml. According to the process of example 1, the magnetic nanoparticles of
25 $\text{Cu}_x\text{Fe}_{1-x}\text{O}\cdot\text{Fe}_2\text{O}_3$ were prepared.

Magnetization of the magnetic nanoparticles and atomic fraction of copper were measured by the process of experimental example 2. And concentration of the magnetic fluids was calculated.

5 The results are shown in Fig. 5.

As shown in Fig. 5, different kind of precipitators had little influence on the concentration of the magnetic fluids but had considerable influence on the magnetization of the magnetic fluids. Particularly, in the case using sodium hydroxide compared with ammonia water, the magnetization of the magnetic nanoparticles decreased while the content of the coprecipitated copper increased. This result obviously corresponds to the one of experimental example 2.

15

<EXPERIMENTAL EXAMPLE 4>

Effect of $(\text{Cu}^{2+}+\text{Fe}^{2+})\text{:Fe}^{3+}$ on magnetization of magnetic particles

Magnetization of the magnetic nanoparticles was measured with different mole ratios of $(\text{Cu}^{2+}+\text{Fe}^{2+})$ to Fe^{3+} by the following experiment. The above $(\text{Cu}^{2+}+\text{Fe}^{2+})$ means to be sum of Cu^{2+} and Fe^{2+} contents in the magnetic nanoparticles of $\text{Cu}_x\text{Fe}_{1-x}\text{O}\cdot\text{Fe}_2\text{O}_3$ prepared in the present invention. The mole ratio of $(\text{Cu}^{2+}+\text{Fe}^{2+})$ to Fe^{3+} is theoretically 1:2. The above ratio is suitable for the preparation of magnetic

nanoparticles in inert gas. However, under the atmosphere, a good magnetization cannot be obtained with the above ratio, due to the oxidation by air.

According to the process of example 1, the magnetic nanoparticles were prepared with different mole ratios of $(\text{Cu}^{2+} + \text{Fe}^{2+})$ to Fe^{3+} . Based on the theoretical value 1:2, the mole ratios of $(\text{Cu}^{2+} + \text{Fe}^{2+})$ to Fe^{3+} were below 1.0:2, 1.1:2, 1.2:2, 1.3:2, 1.4:2 and excess 1.4:2. The magnetization of the magnetic nanoparticles was measured by the process of experimental example 2.

The results are shown in Fig. 6.

As shown in Fig. 6, the magnetization of the magnetic nanoparticles was maximized at the mole ratio of $(\text{Cu}^{2+} + \text{Fe}^{2+}) : \text{Fe}^{3+} = 1.3:2$. Also, in the mole ratio of below 1.0:2 or excess 1.4:2, the magnetic nanoparticles could not be obtained.

Industrial Applicability

As described hereinbefore, the radioactive magnetic fluids of the present invention include the component of Cu^{2+} which radiates a β -ray and γ -ray. Since the γ radiation are easily imaged with γ -camera, the magnetic fluids can be gathered to the treatment site with the radiograph under external magnetic field. Therefore, the radioactive magnetic fluids of the present invention can be

used for treatment of cancer with no side effect and no damage to normal tissue.

Also, decanoic acid and nonanoic acid as surfactants form tightly bonded monomolecular layers around the
5 magnetic nanoparticles, which are comprised in the radioactive magnetic fluids of the present invention. Such surfactants increase particle-particle repulsion and stability of the magnetic fluids. Also, carboxylic acid of the surfactant is exposed to outside, to prevent the
10 magnetic nanoparticles from being oxidized by air.